IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Elzbieta Mietkiewska et al.
Serial No. :	10/596,024
Filed :	05/15/2007
Title :	Fatty Acid Elongase (FAE) Genes and Their Utility in Increasing Erucic Acid and Other Very-Long Chain Fatty Acid Proportions in Seed Oil
Art Unit:	1638
Examiner:	Vinod Kumar
Attorney Docket No.:	PAT 989W-2

Declaration Under 37 C.F.R. Of Dr. David Taylor

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Dr. David C. Taylor declares that:

1. I am co-inventor of one or more claims in U.S. patent application Serial No. 10/596,024 filed May 15, 2007 in the name of Elzbieta Mietkiewska et al. and entitled Fatty Acid Elongase (FAE) Genes and Their Utility in Increasing Erucic Acid and Other Very-Long Chain Fatty Acid Proportions in Seed Oil.

2. I received a B.Sc. in Biochemistry and a Ph.D. in Biology from Carleton University, in Ottawa, Ontario. I actively conduct research in the fields of lipid biochemistry and seed oil biotechnology. During the course of my research, I have been senior author or co-author on more than 80 publications including journals, book chapters, monographs or conference proceedings. I am also an Adjunct Professor in the Department of Plant Science, University of Saskatchewan.

3. I am informed and believe that an Office Action was mailed on or about April 1, 2010, regarding the above-referenced application. I am informed and believe that claims

25-43 were rejected under 35 U.S.C. § 103(a) as assertedly being unpatentable over James et al. (CA 2,203,754 (WO 96/13582) in view of Jaworski et al. (US 20020049994).

4. The literature in connection with fatty acid elongase (FAE) genes contains the following information about performance of various FAE genes.

(a) *Crambe abyssinica* FAE performance:

The *Crambe* FAE gene, which is the subject of the current claims in this application has been further tested by us and the further testing was reported in:

> E Mietkiewska, JM Brost, EM Giblin, DL Barton and DC Taylor (2007) Cloning and functional characterization of the Fatty Acid Elongase 1 (FAE1) gene from high erucic *Crambe abyssinica* cv. Prophet. *Plant Biotechnology Journal*, 5: 636–645.

The *Crambe* FAE gene was expressed in *Brassica carinata*. Expression of the *Crambe* FAE gene in *Brassica carinata* provided an erucic acid change from 35% (wt/wt) in wild type plants to 52% (wt/wt) in the best *Crambe* FAE expression transgenic plants = [best is 17% net]. This represents a 1.5-fold relative increase in erucic acid 22:1.

(b) *Arabidopsis thaliana* FAE performance:

The *Arabidopsis* FAE gene, which is the subject of James et al., has been further tested by us and the further testing was reported in:

> V Katavic, W Friesen, DL Barton, KK Gossen, EM Giblin, T Luciw, J An, J Zou, SL MacKenzie, WA Keller, D Males and DC Taylor (2000) Utility of the Arabidopsis FAE1 and Yeast SLC1-1 Genes for Improvements in Erucic Acid and Oil Content in Rapeseed. *Biochem. Soc. Trans*.28(6): 935-937.
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> V Katavic, W Friesen, DL Barton, KK Gossen, EM Giblin, T Luciw, J An, J Zou, SL MacKenzie, WA Keller, D Males and DC Taylor (2001) Improving erucic acid content in rapeseed through biotechnology: What can the Arabidopsis FAE1 and the Yeast SLC1-1 genes contribute? *Crop Science*, 41: 739-747.

The *Arabidopsis* FAE was expressed in *Brassica napus* cv Hero. Expression of the *Arabidopsis* FAE gene in *Brassica napus* provided an erucic acid change from 43% (wt/wt) in wild type to 48-53% (wt/wt) in the best *Arabidopsis* FAE transgenics = [best is 10% net]. This represents a 1.1-1.2-fold relative increase in erucic acid 22:1.

(c) *Brassica napus* FAE performance:

The *Brassica napus* FAE gene, which is the subject of James et al., has been further tested by us and others and the further testing was reported in:

V Katavic, J-T Zou, C Jako, E-F Marillia and DC Taylor. (2001) Improving Erucic Acid and Oil Content in High Erucic Acid Germplasm: Targets and Strategies. In: Recent Research Developments in Plant Biology, Research SignPost Publishers, Trivandrum, India. pp. 131-142.

V Katavic, E Mietkiewska, DL Barton, EM Giblin, DW Reed and DC Taylor (2002) Restoring Enzyme Activity in Non-functional Low Erucic Acid Brassica napus Fatty Acid Elongase 1 by a Single Amino Acid Substitution. *Eur. J. Biochem.* 269: 5625-5631.

V Katavic, DL Barton, E Mietkiewska and DC Taylor (2004) Improving Very Long Chain Fatty Acid Content in *Brassica* Oilseeds: Studies and Manipulations of Microsomal Elongases. In: Recent Research Dev. Biochem. Vol 5 Research SignPost Publishers, Trivandrum, India pp. 43-52.

Han J, Lühs W, Sonntag K, Zähringer U, Borchardt DS, Wolter FP, Heinz E, Frentzen M (2001) Functional characterization of B-ketoacyl-CoA synthase genes from *Brassica napus* L. *Plant Mol Biol.* 46:229–239

The *Brassica napus* FAE was over-expressed in *Brassica napus* cv Hero. Over-expression of the *Brassica napus* FAE gene in *Brassica napus* provided an erucic acid change from 43% (wt/wt) in wild type to 52% (wt/wt) in the best *Brassica napus* FAE transgenics = [best is 9% net change]. This represents a 1.2-fold relative increase in erucic acid 22:1. It should be noted that Jaworski et al. discloses *Brassica napus* KCS and variants in which nucleotide sequence SEQ ID NO: 33 of Jaworski et al. encoding amino acid sequence SEQ ID NO: 34 is a *Brassica napus* variant differing from wild type *Brassica napus* (SEQ ID NO: 3 encoding SEQ ID NO: 4) by only 1 codon corresponding to the amino acid residue at position 307.

The *Brassica napus* FAE gene has also been further tested by over-expression in *Brassica napus* European HEAR cv providing similar results. This further testing was reported in:

Puyaubert J, Garcia C, Chevalier S, Lessire R (2005) Acyl-CoA elongase, a key enzyme in the development of high-erucic acid rapeseed? *Eur J Lipid Sci Technol.* 107:263–267.

This article further states that "Nonetheless, manipulations affecting exclusively the gene encoding the 3-ketoacyl-CoA synthase were not sufficient to increase the erucic acid content in seed oil." page 265.

(d) *Brassica juncea* FAE performance:

The *Brassica juncea* FAE gene has also been further tested, the further testing reported in:

> Kanrar, S., Venkateswari, J., Dureja, P., Kirti, P.B. and Chopra, V.L. (2006) Modification of erucic acid content in Indian mustard (*Brassica juncea*) by up-regulation and down-regulation of the *Brassica juncea* fatty acid elongation1 (BjFAE1) gene. *Plant Cell Rep.* 25, 148--155

The *Brassica juncea* FAE was over-expressed in *Brassica juncea*. Over-expression of the *Brassica juncea* FAE gene in *Brassica juncea* provided an erucic acid change from 36% (wt/wt) in wild type to 37-45% (wt/wt) in the best *Brassica juncea* FAE transgenics = [best is 9% net change]. This represents a 1.25-fold relative increase in erucic acid 22:1.

5. Expression of the *Crambe* FAE results in a significantly greater relative increase in erucic acid 22:1 levels than expression or over-expression of the other FAEs as summarized in the Table A below. Despite the various FAEs being extremely high in terms of homology at the amino acid level, their actual performance in biotech experiments/applications wherein they are ectopically or over-expressed is variable, and one would never have predicted that the *Crambe* FAE would perform better than others.

Table A

FAE	Reference	Relative increase in erucic acid 22:1
Crambe	Present invention - SEQ ID NO: 25 encoding SEQ ID NO: 24	1.5 fold
Arabidopsis	James et al. - SEQ ID NO: 1 encoding SEQ ID NO: 2 Jaworski et al. - SEQ ID NO: 1 encoding SEQ ID NO: 2	1.1 to 1.2 fold
B. napus	Jaworski et al. - SEQ ID NO: 3 encoding SEQ ID NO: 4	1.2 fold
B. juncea		1.25 fold

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Dr. David C. Taylor

Date: May 20, 2010